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ARTICLE

Emerging citrus diseases in Europe caused by species of *Diaporthe*

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Abstract: Species of *Diaporthe* are considered important plant pathogens, saprobes, and endophytes on a wide range of plant hosts. Several species are well-known on citrus, either as agents of pre- or post-harvest infections, such as dieback, melanose and stem-end rot on fruit. In this study we explored the occurrence, diversity and pathogenicity of *Diaporthe* species associated with *Citrus* and allied genera in European orchards, nurseries, and gardens. Surveys were carried out during 2015 and 2016 in Greece, Italy, Malta, Portugal, and Spain. A total of 79 *Diaporthe* strains were isolated from symptomatic twigs, branches and trunks. A multi-locus phylogeny was established based on five genomic loci (ITS, *tef1*, *cal*, *his3* and *tub2*), and the morphological characters of the isolates determined. Preliminary pathogenicity tests were performed on lemon, lime, and orange plants with representative isolates. The most commonly isolated species were *D. foeniculina* and *D. baccae*, while only four isolates of *D. novem* were collected. Two new *Diaporthe* species, described here as *D. limonicola* and *D. melitensis* spp. nov. were found associated with a new devastating dieback disease of lemon plants. Furthermore, one cluster of sterile *Diaporthe* isolates was renamed as *D. infertilis*. Pathogenicity tests revealed most of the *Citrus* species as susceptible to *D. baccae*, *D. foeniculina,* and *D. novem*. Moreover, *D. limonicola* and *D. melitensis* caused serious cankers affecting all the *Citrus* species tested. This study is the first report of *D. baccae* and *D. novem* on citrus in Europe, and the first detection of a new *Diaporthe* canker disease of citrus in Europe. However, no isolates of *D. citri* were found. The study improves our understanding of the species associated with several disease symptoms on citrus plants, and provides useful information for effective disease management.

Key words:
Canker
Citrus
multi-locus sequence typing
pathogenicity

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INTRODUCTION

Diaporthe species are present worldwide as plant pathogens and endophytes in healthy leaves, stems, seeds and roots, or as saprobes on decaying tissues of a wide range of hosts (Muralli *et al*. 2006, Garcia-Reyne *et al.* 2011, Udayanga *et al*. 2011). *Diaporthe* species are well-known as the causal agents of many important plant diseases, including root and fruit rots, dieback, stem cankers, leaf spots, leaf and pod blights, and seed decay (Uecker 1988, Mostert *et al.* 2001a, b, Van Rensburg *et al.* 2006, Rehner & Uecker 1994, Santos *et al.* 2011, Udayanga *et al.* 2011, Diaz *et al*. 2017). Species of *Diaporthe* have also been extensively screened in bioassays for natural products (Isaka *et al.* 2001, Dai *et al*. 2005, Kumaran & Hur 2009, Yang *et al*. 2010), and for the biocontrol of fungal pathogens (Santos *et al.* 2016).

The generic names *Diaporthe* and *Phomopsis* are no longer used to distinguish different morphs of this genus, and recent studies (Rossman *et al*. 2015) have recommended that *Diaporthe* be adopted as the correct generic name as it has priority over *Phomopsis*.

Diaporthe was historically considered monophyletic based on the typical *Phomopsis* asexual morph and diaporthalean sexual morph (Gomes *et al.* 2013). However, the paraphyletic nature was recently revealed by Gao *et al*. (2017), who demonstrated that *Ophiodiaporthe* (Fu *et al.* 2013), *Pustulomyces* (Dai *et al.* 2014), *Phaeocytostroma,* and *Stenocarpella* (Lamprecht *et al.* 2011), are embedded in *Diaporthe s. lat*. To address this issue, Senanayake *et al.* (2017) subsequently named several additional diaporthe-like clades within *Diaporthales*.

The taxonomy of *Diaporthe* species has been reviewed in several major studies (Thompson *et al*. 2011, 2014, Gomes *et al.* 2013, Udayanga *et al.* 2014a, b, 2015). Almost 2000 species names are available for both *Diaporthe* and *Phomopsis* (Index Fungorum; http://www.indexfungorum.org). The majority of the known species in early literature were described in relation to their host association (Uecker 1988), except for about 150 species that have been described more recently supported by molecular data (Gomes *et al*. 2013, Lombard *et al.* 2014, Udayanga *et al.* 2014a, b, 2015). However, most *Diaporthe* species can be found on

diverse hosts, and can co-occur on the same host or lesion in different life modes (Rehner & Uecker 1994, Mostert *et al.* 2001a, Guarnaccia *et al.* 2016). This is demonstrated by *D. foeniculina*, usually known as an opportunistic pathogen of various herbaceous weeds, ornamentals, and fruit trees including citrus (Santos & Phillips 2009, Udayanga *et al.* 2014b). However, it has also been isolated from tropical trees as an endophyte, and from herbaceous plants and weeds as a pathogen or saprobe (Udayanga *et al.* 2014a). As a consequence, identification and description of species based on host association alone is no longer tenable within *Diaporthe* (Gomes *et al*. 2013, Udayanga *et al.* 2014a, b).

Before the molecular era, morphological characters such as immersed ascomata and erumpent pseudostroma with elongated perithecial necks in the sexual morph (Udayanga *et al*. 2011), and black conidiomata with dimorphic conidia in the asexual morph (Rehner & Uecker 1994), was the basis on which to study the taxonomy of *Diaporthe* (Van der Aa *et al*. 1990). Recent studies demonstrated that these characters are not always reliable for species level identification due to their variability under changing environmental conditions (Gomes *et al*. 2013).

Following the adoption of DNA sequence-based methods, the polyphasic protocols for studying the genus significantly changed the classification and species concepts, resulting in a rapid increase in the description of novelties. Therefore, genealogical concordance methods, based on multi-gene DNA sequence data, provide a much clearer approach to resolving the taxonomy for *Diaporthe*.

Recent plant pathological studies have shown several *Diaporthe* species to be particularly important on a wide range of economically significant agricultural crops, such as blueberries, citrus, grapes, oaks, sunflowers, soybeans, tea plants, tropical fruits, vegetables, and various trees (Van Rensburg *et al.* 2006, Crous *et al*. 2011a, b, 2016, Thompson *et al.* 2011, Santos & Phillips 2009, Santos *et al*. 2011, Grasso *et al*. 2012, Huang *et al*. 2013, Lombard *et al*. 2014, Gao *et al.* 2015, 2016, Udayanga *et al.* 2015, Guarnaccia *et al.* 2016). Furthermore, several *Citrus* species are colonized and/or affected by different *Diaporthe* species (Timmer *et al.* 2000, Huang *et al*. 2013), which are focussed on here.

BACKGROUND

Citrus represents one of the most important fruit industries worldwide. In the Mediterranean region, Greece, Italy, Portugal, and Spain especially are important producers of citrus fruits, and are the biggest fruit exporter after South Africa (FAO 2016). Therefore, recognizing the pathogens affecting these crops in these countries is imperative.

Diaporthe citri is a well-known pathogen causing melanose and stem-end rot disease of *Citrus* species in several regions (Timmer 2000, Mondal *et al.* 2007). Several additional *Diaporthe* species have been reported associated with *Citrus* (often as *Phomopsis*) and have previously been considered as synonyms of *D. citri*, such as *D. citrincola* described from the Philippines, *P. californica* from California, *P. caribaea* from Cuba, and *P. cytosporella* from Italy (Rehm 1914, Fawcett 1922). Wehmeyer (1933) also considered *D. medusaea*, *D. californica*, *P. citri,* and *P. citrincola* as synonyms of *Diaporthe citri*.

Polyphasic approaches in recent years have revealed many species associated with citrus. Huang *et al*. (2013) reported *D. citri* as the predominant species in China and described two new taxa: *D. citriasiana* and *D. citrichinensis*. In another study, Huang *et al.* (2015) identified several *Diaporthe* species as endophytes of citrus but which had previously been recovered from other hosts, such as *D. endophytica*, *D. eres*, *D. hongkongensis*, *D. sojae*, and the different taxa clustering in the *D. arecae* species complex. Moreover, they described *D. biconispora*, *D. biguttulata*, *D. discoidispora*, *D. multigutullata*, *D. ovalispora*, *D. subclavata*, and *D. unshiuensis* as new species occurring on citrus. Several strains from China, Korea, New Zealand, and the USA have been re-assessed by Udayanga *et al*. (2014b) within *D. citri*, which was also epitypified. In the same study, *D. cytosporella* was recovered from specimens of *Citrus limon*, *C. limonia,* and *C. sinensis* collected respectively in Spain, Italy, and the USA, and *D. foeniculina* has also been widely associated with citrus.

Diaporthe citri is generally accepted as an important pathogen of citrus, causing stem-end rot and melanose of fruits, young leaf and shoot gummosis, and blight of perennial branches and trunks (Kucharek *et al.* 1983, Timmer & Kucharek 2001, Mondal *et al.* 2007, Udayanga *et al.* 2014b). This species occurs in many citrus growing regions of the world on several *Citrus* species, including *C. limon*, *C. paradisi*, *C. reticulata,* and *C. sinensis* (Timmer *et al.* 2000).

Further infections involving twigs, perennial branches and trunks of citrus are caused by other *Diaporthe* species, such as cankers developing in woody tissues, often with a gummose exudate, generating serious blight and dieback (Huang *et al*. 2013, Mahadevakumar *et al.* 2014). Canker diseases of citrus are also caused by other fungal genera such as *Fusarium* and *Neocosmospora* (Sandoval-Denis *et al*. 2018), and species of *Botryosphaeriaceae* and *Diatrypaceae* (Timmer *et al*. 2000, Polizzi *et al.* 2009, Mayorquin *et al*. 2016).

Although the biology and epidemiology of melanose are well studied also with a robust phylogenetic relationship of the causal organisms, genetic variability and population structure (Burnett 1962, Mondal *et al.* 2004, 2007, Udayanga *et al*. 2014b), the identification of *Diaporthe* species associated with citrus cankers and dieback has not been well resolved. Moreover, Gomes *et al*. (2013) performed a major phylogenetic and morphological study of *Diaporthe* species and grouped three isolates, one of which was collected from *Citrus sinensis* in Suriname, under *D. citri*. However, Udayanga *et al*. (2014b) re-assessed *D. citri* based on molecular phylogenetic analysis of conserved ex-type and additional strains collected exclusively from symptomatic citrus tissues in different geographic locations worldwide. Furthermore, according to this latter study, *D. citri* is unknown in Europe. Because of all these findings, changes in species concepts and poor investigation of *Diaporthe* on citrus in Europe, new surveys were required to study *Diaporthe* species diversity related to citrus and their occurrence and association with diseases.

The current study aims to investigate the major citrus production areas in Europe by employing large-scale

sampling to isolate *Diaporthe* strains, and to identify the strains obtained in the light of modern taxonomic concepts *via* morphological characterization and multi-locus DNA sequence data. In 2015 and 2016, several surveys were conducted in commercial nurseries, citrus orchards, gardens, backyards, and plant collections to determine the occurrence of *Diaporthe* species associated with *Citrus* and allied genera (e.g. *Microcitrus*). In particular the objectives of the present study were to: (1) conduct extensive surveys for sampling symptomatic plant materials; (2) cultivate as many *Diaporthe* isolates as possible; (3) subject those isolates to DNA sequence analyses combined with morphological characterization; (4) compare the obtained results with the data from other phylogenetic studies on the genus; (5) place three strains previously named as *D. citri* in the correct taxonomic context based on DNA sequence inference; and (6) evaluate the pathogenicity of the isolated *Diaporthe* species to citrus plants.

MATERIALS AND METHODS

Sampling and isolation

During 2015 and 2016 many regions of the main citrus-producing area of Europe were surveyed (Guarnaccia *et al*. 2017a, b). Twig, branch and trunk portions showing cankers and dieback were collected from more than 90 sites in: Andalusia, Valencia, and the Balearic Islands (Spain); Apulia, Calabria, Sicily, and the Aeolian Islands (Italy); Algarve (Portugal); Arta, Crete, Missolonghi, and Nafplio (Greece); and Malta and Gozo (Malta). Investigated species of *Citrus* and allied genera such as *Microcitrus* (*Rutaceae*) included Australasian lime, citrons, kumquat, mandarins, oranges, pumelo, grapefruit, limes, and lemons.

Wood fragments (5 × 5 mm) were cut from the margin between affected and healthy tissues and washed in running tap water. Then, each fragment was surface sterilised by soaking in 70 % ethanol for 5 s, 4 % sodium hypochlorite for 90 s, sterile water for 60 s (Kumaresan & Suryanarayanan 2001) and then dried on sterile filter paper. The fragments were placed on malt extract agar (MEA; Crous *et al*. 2009) amended with 100 µg / mL penicillin and 100 µg / mL streptomycin (MEA-PS) and incubated at 25 °C until characteristic *Diaporthe* colonies were observed. In a second procedure, plant material was incubated in moist chambers at room temperature (20 ± 3 °C) for up to 10 d and inspected daily for fungal sporulation. Sporulating conidiomata obtained through both procedures were collected and crushed in a drop of sterile water and then spread over the surface of MEA-PS plates. After 24 h germinating spores were individually transferred onto MEA plates. The isolates used in this study are maintained in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands, and in the working collection of Pedro Crous (CPC), housed at the Westerdijk Institute.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted using a Wizard® Genomic DNA Purification Kit (Promega, WI) following the manufacturer's instructions. Partial regions of six loci were amplified. The primers ITS5 and ITS4 (White *et al*. 1990) were used to amplify the ITS region of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA, the first internal transcribed spacer region, the 5.8S rRNA gene; the second internal transcribed spacer region and the 5' end of the 28S rRNA gene. The primers EF1-728F and EF1-986R (Carbone & Kohn 1999) were used to amplify part of the translation elongation factor 1-α gene (*tef1*). Primers CAL-228F and CAL-737R (Carbone & Kohn 1999) or CL1/ CL2A (O'Donnell *et al*. 2000) were used to amplify part of the calmodulin (*cal*) gene. The partial histone H3 (*his3*) region was amplified using CYLH3F and H3-1b primer sets (Glass & Donaldson 1995, Crous *et al*. 2004a), and the beta-tubulin (*tub2*) region was amplified using Bt2a and Bt2b primer sets (Glass & Donaldson 1995). The PCR products were sequenced in both directions using the BigDye® Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems Life Technologies, Carlsbad, CA), after which amplicons were purified through Sephadex G-50 Fine columns (GE Healthcare, Freiburg) in MultiScreen HV plates (Millipore, Billerica, MA). Purified sequence reactions were analysed on an Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA). The DNA sequences generated were analysed and consensus sequences were computed using SeqMan Pro (DNASTAR, Madison, WI).

Phylogenetic analyses

New sequences generated in this study were blasted against the NCBI's GenBank nucleotide database to determine the closest relatives for a taxonomic framework of the studied isolates. Alignments of different gene regions, including sequences obtained from this study and sequences downloaded from GenBank, were initially performed with the MAFFT v. 7 online server (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh & Standley 2013), and then manually adjusted in MEGA v. 7 (Kumar *et al*. 2016).

To establish the identity of the isolates at species level, phylogenetic analyses were conducted first individually for each locus (data not shown) and then as combined analyses of five loci. One analysis was performed for all the *Diaporthe* isolates recovered from samples collected during the surveys conducted for this study. Additional reference sequences were selected based on recent studies of *Diaporthe* species (Gomes *et al*. 2013, Huang *et al*. 2013, Udayanga *et al*. 2014a, b). Phylogenetic analyses were based on Maximum Parsimony (MP) for all the individual loci and on both MP and Bayesian Inference (BI) for the multi-locus analyses. For BI, the best evolutionary model for each partition was determined using MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses. MrBayes v. 3.2.5 (Ronquist *et al*. 2012) was used to generate phylogenetic trees under optimal criteria per partition. The Markov Chain Monte Carlo (MCMC) analysis used four chains and started from a random tree topology. The heating parameter was set to 0.2 and trees were sampled every 1000 generations. Analyses stopped once the average standard deviation of split frequencies was below 0.01. The MP analyses were done using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10; Swofford 2003). Phylogenetic relationships were estimated by heuristic searches with 100

random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on "best trees" only with all characters weighted equally and alignment gaps treated as fifth state. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistence index (RC) were calculated for parsimony and the bootstrap analyses (Hillis & Bull 1993) were based on 1000 replications. Sequences generated in this study are deposited in GenBank (Table 1) and alignments and phylogenetic trees in TreeBASE (www.treebase.org).

Morphological analyses

Agar plugs (6 mm diam) were taken from the edge of actively growing cultures on MEA and transferred onto the centre of 9 cm diam Petri dishes containing 2 % tap water agar supplemented with sterile pine needles (PNA; Smith *et al*. 1996), potato dextrose agar (PDA), oatmeal agar (OA) and MEA (Crous *et al*. 2009), and incubated at 20–21 °C under a 12 h near-ultraviolet light/12 h dark cycle to induce sporulation as described in recent studies (Gomes *et al*. 2013, Lombard *et al.* 2014). Colony characters and pigment production on MEA, OA and PDA were noted after 10 d. Colony colours were rated according to Rayner (1970). Cultures were examined periodically for the development of ascomata and conidiomata. Colony diameters were measured after 7 and 10 d. The morphological characteristics were examined by mounting fungal structures in clear lactic acid and 30 measurements at ×1000 magnification were determined for each isolate using a Zeiss Axioscope 2 microscope with interference contrast (DIC) optics. Descriptions, nomenclature and illustrations of taxonomic novelties are deposited in MycoBank (www.MycoBank.org; Crous *et al*. 2004b).

Pathogenicity

Pathogenicity tests with five *Diaporthe* species isolated from the European citrus samples were performed to satisfy Koch's postulates.

Two isolates of each of the five species (*D. baccae*: CPC 26170, CPC 27831; *D. foeniculina*: CPC 28033, CPC 28081; *D. limonicola*: CPC 28200, CPC 31137; *D. melitensis*: CPC 27873, CPC 27875; and *D. novem*: CPC 26188, CPC 28165), were inoculated onto potted 2-yr-old healthy plants of lemon (*Citrus limon*), lime (*C. aurantiifolia*), mandarin (*C. reticulata*), and two clones ('New Hall' and 'Tarocco Meli') of sweet orange (*C. sinensis*). Three plants per replicate for each isolate were inoculated, each having five wounds on twigs made using a sterile blade. Mycelial plugs (6 mm diam), taken from the margin of actively growing colonies on MEA, were placed on the wound sites on each plant. An equivalent number of plants and inoculation sites were inoculated with sterile MEA plugs and served as controls. The inoculation sites were covered with Parafilm® (American National Can, Chicago, IL). The inoculated plants were incubated with a 16 h photoperiod in a growth chamber at 100 % relative humidity and 25 ± 1 °C. After 2 mo external symptoms were assessed. Twigs were cut and the bark peeled off to check for any internal discolouration.

Small sections (0.5 cm) of symptomatic tissue from the edge of twig lesions were placed on MEA to re-isolate the fungal species, and were identified based on *tef1* and *tub2* sequencing to fulfil Koch's postulates.

RESULTS

Isolates

Several shoot blight and canker infections on woody tissue were frequently observed on multiple *Citrus* species in all countries investigated. Some orchards presented blight of vigorously growing branches and cankers involving both scion branches and rootstock trunks, resulting in a general dieback and tree death (Fig. 1A). Affected trunks and branches appeared cracked, darkly discoloured and/or slightly sunken. Abundant gummosis was frequently associated with the affected tissues (Fig. 1B–D). Twigs showed wilting, typical dieback and wither-tip, and occasionally gummosis (Fig. 1E–F). Under the bark, cankers were reddish brown and variable in shape. Pycnidial formation on dead twig tissue was observed (Fig. 1G). A total of 79 monosporic isolates resembling those of the genus *Diaporthe* were collected. The *Diaporthe* isolates were recovered from 10 species of *Citrus* at 31 sites in different locations of Greece, Italy, Malta, Spain, and Portugal. Among them, 27 isolates were obtained from branch infections, 13 were associated with trunk cankers, and 39 from twig dieback (Table 1).

Phylogenetic analyses

Six alignments were analysed representing single gene analyses of ITS, *tub2*, *his3*, *tef1*, *cal* and a combined alignment of the five genes. The alignments produced topologically similar trees. The combined species phylogeny of the *Diaporthe* isolates consisted of 123 sequences, including the outgroup sequences of *Diaporthella corylina* (culture CBS 121124). A total of 3026 characters (ITS: 1–582, *tef1*: 589–1052, *tub2*: 1059–1 862, *cal*: 1869–2484, *his3*: 2 491–3026) were included in the phylogenetic analysis, 1355 characters were parsimony-informative, 468 were variable and parsimony-uninformative, and 1161 were constant. A maximum of 1000 equally most parsimonious trees were saved (Tree length = 5528, CI = 0.584, RI = 0.868 and RC = 0.507). Bootstrap support values from the parsimony analysis are plotted on the Bayesian phylogenies in Fig. 2. For the Bayesian analyses, MrModeltest suggested that all partitions should be analysed with dirichlet state frequency distributions. The following models were recommended by MrModeltest and used: GTR+I+G for ITS, *tef1* and *cal*, HKY+G for *tub2* and GTR+G for *his3*. In the Bayesian analysis, the ITS partition had 188 unique site patterns, the *tef1* partition had 357 unique site patterns, the *tub2* partition had 510 unique site patterns, the *cal* partition had 364 unique site patterns, the *his3* partition had 239 unique site patterns and the analysis ran for 1 880 000 generations, resulting in 3762 trees of which 2822 trees were used to calculate the posterior probabilities.

In the combined analysis, 54 *Citrus* isolates clustered with five reference strains and the ex-type of *D. foeniculina*, whilst 14 isolates clustered with the ex-type of *D. baccae*. Four isolates clustered with the ex-type strain of *D. novem*. Moreover, five isolates identified as *D. limonicola* and a further two as *D. melitensis*, formed two highly supported subclades (1.00/100) embedded in the *D. arecae* species complex.

The individual alignments and trees of the five single loci used in the analyses, were also compared with respect to their performance in species recognition. *D. novem* was differentiated by each gene used. Moreover, *tef1* and *tub2* separated both *D. limonicola* and *D. melitensis* from the other species belonging to the *D. arecae* species complex.

Fig. 1. Symptoms on citrus tissues with associated *Diaporthe* species. **A.** Commercial lemon orchard infected by *D. limonicola* and *D. melitensis* (Malta). **B**–**C.** Trunk canker with gummosis of *Citrus limon* and *C. sinensis* plants (Malta). **D.** Branch canker of *C. sinensis* (Portugal). **E**–**F.** Twigs dieback of lemon (Italy). **G.** Orange twigs wither-tip with *Diaporthe* pycnidial formation (Italy).

Table 1. Collection details and GenBank accession numbers of isolates included in this study.

Species	Culture no.[1]	Host	Locality	Associated symptoms	GenBank no.[2]				
					ITS	*tub2*	*his3*	*tef1*	*cal*
D. angelicae	**CBS 111592**	*Heracleum sphondylium*	Austria	-	KC343026	KC343994	KC343511	KC343752	KC343268
D. arecae	**CBS 161.64**	*Areca catechu*	India	-	KC343032	KC344000	KC343516	KC343758	KC343274
	CBS 535.75	*Citrus* sp.	Suriname	-	KC343033	KC344001	KC343517	KC343759	KC343275
D. arengae	**CBS 114979**	*Arenga engleri*	Hong Kong	-	KC343034	KC344002	KC343518	KC343760	KC343276
D. baccae	**CBS 136972**	*Vaccinium corymbosum*	Italy	-	KJ160565	*MF418509*	*MF418264*	KJ160597	-
	CPC 26170 = CBS 142545	*Citrus sinensis* 'Tarocco Tapi'	Italy, Catania	Twig dieback	*MF418351*	*MF418510*	*MF418265*	*MF418430*	*MF418185*
	CPC 26465	*Citrus limon*	Italy, Catania	Branch canker	*MF418352*	*MF418511*	*MF418266*	*MF418431*	*MF418186*
	CPC 26963	*Citrus paradisi*	Italy, Vibo Valentia	Branch canker	*MF418353*	*MF418512*	*MF418267*	*MF418432*	*MF418187*
	CPC 27029	*Citrus sinensis*	Italy, Vibo Valentia	Twig dieback	*MF418354*	*MF418513*	*MF418268*	*MF418433*	*MF418188*
	CPC 27075	*Citrus limon*	Italy, Vibo Valentia	Twig dieback	*MF418355*	*MF418514*	*MF418269*	*MF418434*	*MF418189*
	CPC 27079	*Citrus limon*	Italy, Vibo Valentia	Twig dieback	*MF418356*	*MF418515*	*MF418270*	*MF418435*	*MF418190*
	CPC 27821	*Citrus reticulata* 'Caffin'	Italy, Cosenza	Trunk canker	*MF418357*	*MF418516*	*MF418271*	*MF418436*	*MF418191*
	CPC 27831 = CBS 142546	*Citrus sinensis*	Italy, Catania	Trunk canker	*MF418358*	*MF418517*	*MF418272*	*MF418437*	*MF418192*
	CPC 27834	*Citrus sinensis*	Italy, Catania	Trunk canker	*MF418359*	*MF418518*	*MF418273*	*MF418438*	*MF418193*
	CPC 27835	*Citrus sinensis*	Italy, Catania	Trunk canker	*MF418360*	*MF418519*	*MF418274*	*MF418439*	*MF418194*
	CPC 27836	*Citrus sinensis*	Italy, Catania	Trunk canker	*MF418361*	*MF418520*	*MF418275*	*MF418440*	*MF418195*
	CPC 27837	*Citrus sinensis*	Italy, Catania	Trunk canker	*MF418362*	*MF418521*	*MF418276*	*MF418441*	*MF418196*
	CPC 27850	*Citrus sinensis*	Italy, Catania	Twig dieback	*MF418363*	*MF418522*	*MF418277*	*MF418442*	*MF418197*
	CPC 27852	*Citrus sinensis*	Italy, Catania	Twig dieback	*MF418364*	*MF418523*	*MF418278*	*MF418443*	*MF418198*
D. biconispora	**ICMP20654**	*Citrus grandis*	China	-	KJ490597	KJ490418	KJ490539	KJ490476	-
D. biguttulata	**ICMP20657**	*Citrus limon*	China	-	KJ490582	KJ490403	KJ490524	KJ490461	-
D. citri	CBS 134237	*Citrus reticulata*	China	-	JQ954660	KC357426	*MF418279*	JQ954676	KC357465
	CBS 134239	*Citrus sinensis*	Florida, USA	-	KC357553	KC357456	*MF418280*	KC357522	KC357488
	CBS 135422	*Citrus* sp.	USA	-	KC843311	KC843187	*MF418281*	KC843071	KC843157
D. citriasiana	**CBS 134240**	*Citrus unshiu*	China	-	JQ954645	KC357459	*MF418282*	JQ954663	KC357491
D. citrichinensis	**CBS 134242**	*Citrus* sp.	China	-	JQ954648	*MF418524*	KJ420880	JQ954666	KC357494
D. cuppatea	**CBS 117499**	*Aspalathus linearis*	South Africa	-	AY339322	JX275420	KC343541	AY339354	JX197414
D. cytosporella	**CBS 137020**	*Citrus limon*	Spain	-	KC843307	KC843221	*MF418283*	KC843116	KC843141
D. discoidispora	**ICMP20662**	*Citrus unshiu*	China	-	KJ490624	KJ490445	KJ490566	KJ490503	-
D. endophytica	ZJUD73	*Citrus unshiu*	China	-	KJ490608	KJ490429	KJ490550	KJ490487	-
D. eres	**CBS 439.82**	*Cotoneaster* sp.	Scotland	-	KC343090	KC344058	KC343574	KC343816	KC343332
D. foeniculina	CBS 187.27	*Camellia sinensis*	Italy	-	KC343107	KC344075	KC343591	KC343833	KC343349
	CBS 111553	*Foeniculum vulgare*	Spain	-	KC343101	KC344069	KC343585	KC343827	KC343343

Table 1. (Continued).

Species	Culture no.[1]	Host	Locality	Associated symptoms	GenBank no.[2]				
					ITS	*tub2*	*his3*	*tef1*	*cal*
	CBS 111554	*Foeniculum vulgare*	Portugal	-	KC343102	KC344070	KC343586	KC343828	KC343344
	CBS 123208	*Foeniculum vulgare*	Portugal	-	KC343104	KC344072	KC343588	KC343830	KC343346
	CBS 123209	*Foeniculum vulgare*	Portugal	-	KC343105	KC344073	KC343589	KC343831	KC343347
	CBS 135430	*Citrus limon*	USA	-	KC843301	KC843215	*MF418284*	KC843110	KC843135
	CPC 26184	*Citrus maxima*	Italy, Messina	Branch canker	*MF418365*	*MF418525*	*MF418285*	*MF418444*	*MF418199*
	CPC 26194	*Citrus sinensis* 'Sanguinello'	Italy, Messina	Branch canker	*MF418366*	*MF418526*	*MF418286*	*MF418445*	*MF418200*
	CPC 26365	*Citrus limon*	Italy, Catania	Twig dieback	*MF418367*	*MF418527*	*MF418287*	*MF418446*	*MF418201*
	CPC 26439	*Citrus reticulata*	Italy, Catania	Twig dieback	*MF418368*	*MF418528*	*MF418288*	*MF418447*	*MF418202*
	CPC 26441	*Citrus reticulata*	Italy, Catania	Twig dieback	*MF418369*	*MF418529*	*MF418289*	*MF418448*	*MF418203*
	CPC 26461	*Citrus reticulata*	Italy, Catania	Twig dieback	*MF418370*	*MF418530*	*MF418290*	*MF418449*	*MF418204*
	CPC 26863	*Citrus maxima*	Greece, Missolonghi	Branch canker	*MF418371*	*MF418531*	*MF418291*	*MF418450*	*MF418205*
	CPC 26873	*Citrus reticulata*	Greece, Arta	Twig dieback	*MF418372*	*MF418532*	*MF418292*	*MF418451*	*MF418206*
	CPC 26883	*Citrus maxima*	Greece, Missolonghi	Branch canker	*MF418373*	*MF418533*	*MF418293*	*MF418452*	*MF418207*
	CPC 26885	*Citrus bergamia*	Greece, Missolonghi	Branch canker	*MF418374*	*MF418534*	*MF418294*	*MF418453*	*MF418208*
	CPC 26913	*Citrus limon*	Greece, Missolonghi	Branch canker	*MF418375*	*MF418535*	*MF418295*	*MF418454*	*MF418209*
	CPC 26923	*Citrus maxima*	Greece, Missolonghi	Branch canker	*MF418376*	*MF418536*	*MF418296*	*MF418455*	*MF418210*
	CPC 26927	*Citrus maxima*	Greece, Missolonghi	Branch canker	*MF418377*	*MF418537*	*MF418297*	*MF418456*	*MF418211*
	CPC 26953	*Citrus bergamia*	Greece, Missolonghi	Branch canker	*MF418378*	*MF418538*	*MF418298*	*MF418457*	*MF418212*
	CPC 26967	*Citrus mitis*	Italy, Messina	Twig dieback	*MF418379*	*MF418539*	*MF418299*	*MF418458*	*MF418213*
	CPC 26971	*Citrus mitis*	Italy, Messina	Twig dieback	*MF418380*	*MF418540*	*MF418300*	*MF418459*	*MF418214*
	CPC 27027	*Citrus limon*	Italy, Cosenza	Branch canker	*MF418381*	*MF418541*	*MF418301*	*MF418460*	*MF418215*
	CPC 27033	*Citrus mitis*	Italy, Messina	Twig dieback	*MF418382*	*MF418542*	*MF418302*	*MF418461*	*MF418216*
	CPC 27037	*Citrus paradisi*	Italy, Vibo Valentia	Branch canker	*MF418383*	*MF418543*	*MF418303*	*MF418462*	*MF418217*
	CPC 27041	*Citrus sinensis*	Italy, Cosenza	Branch canker	*MF418384*	*MF418544*	*MF418304*	*MF418463*	*MF418218*
	CPC 27167	*Citrus paradisi*	Italy, Vibo Valentia	Branch canker	*MF418385*	*MF418545*	*MF418305*	*MF418464*	*MF418219*
	CPC 27756	*Citrus limon*	Italy, Catania	Trunk canker	*MF418386*	*MF418546*	*MF418306*	*MF418465*	*MF418220*
	CPC 27832	*Citrus sinensis*	Italy, Catania	Trunk canker	*MF418387*	*MF418547*	*MF418307*	*MF418466*	*MF418221*
	CPC 27833	*Citrus sinensis*	Italy, Catania	Trunk canker	*MF418388*	*MF418548*	*MF418308*	*MF418467*	*MF418222*
	CPC 27859	*Citrus paradisi*	Malta, Gozo	Trunk canker	*MF418389*	*MF418549*	*MF418309*	*MF418468*	*MF418223*
	CPC 27877	*Citrus limon*	Malta, Gozo	Trunk canker	*MF418390*	*MF418550*	*MF418310*	*MF418469*	*MF418224*
	CPC 27895	*Citrus japonica*	Malta, Gozo	Twig dieback	*MF418391*	*MF418551*	*MF418311*	*MF418470*	*MF418225*
	CPC 27896	*Citrus japonica*	Malta, Gozo	Twig dieback	*MF418392*	*MF418552*	*MF418312*	*MF418471*	*MF418226*

Table 1. (Continued).

Species	Culture no.[1]	Host	Locality	Associated symptoms	GenBank no.[2]				
					ITS	*tub2*	*his3*	*tef1*	*cal*
	CPC 27897	*Citrus japonica*	Malta, Gozo	Twig dieback	*MF418393*	*MF418553*	*MF418313*	*MF418472*	*MF418227*
	CPC 27898	*Citrus japonica*	Malta, Gozo	Twig dieback	*MF418394*	*MF418554*	*MF418314*	*MF418473*	*MF418228*
	CPC 27901	*Citrus limon*	Malta, Gozo	Branch canker	*MF418395*	*MF418555*	*MF418315*	*MF418474*	*MF418229*
	CPC 27903	*Citrus limon*	Malta, Gozo	Branch canker	*MF418396*	*MF418556*	*MF418316*	*MF418475*	*MF418230*
	CPC 27945	*Citrus paradisi*	Portugal, Faro	Branch canker	*MF418397*	*MF418557*	*MF418317*	*MF418476*	*MF418231*
	CPC 27947	*Citrus sinensis*	Portugal, Faro	Branch canker	*MF418398*	*MF418558*	*MF418318*	*MF418477*	*MF418232*
	CPC 27949	*Citrus sinensis*	Portugal, Faro	Branch canker	*MF418399*	*MF418559*	*MF418319*	*MF418478*	*MF418233*
	CPC 27950	*Citrus sinensis*	Portugal, Faro	Twig dieback	*MF418400*	*MF418560*	*MF418320*	*MF418479*	*MF418234*
	CPC 27959	*Citrus sinensis*	Portugal, Faro	Twig dieback	*MF418401*	*MF418561*	*MF418321*	*MF418480*	*MF418235*
	CPC 28033 = CBS 142547	*Citrus sinensis* 'Valencia'	Portugal, Mesquita	Twig dieback	*MF418402*	*MF418562*	*MF418322*	*MF418481*	*MF418236*
	CPC 28035	*Citrus paradisi*	Portugal, Faro	Twig dieback	*MF418403*	*MF418563*	*MF418323*	*MF418482*	*MF418237*
	CPC 28039	*Citrus limon*	Portugal, Monchique	Twig dieback	*MF418404*	*MF418564*	*MF418324*	*MF418483*	*MF418238*
	CPC 28041	*Citrus limon*	Portugal, Monchique	Twig dieback	*MF418405*	*MF418565*	*MF418325*	*MF418484*	*MF418239*
	CPC 28043	*Citrus limon*	Portugal, Monchique	Twig dieback	*MF418406*	*MF418566*	*MF418326*	*MF418485*	*MF418240*
	CPC 28045	*Citrus limon*	Portugal, Monchique	Twig dieback	*MF418407*	*MF418567*	*MF418327*	*MF418486*	*MF418241*
	CPC 28047	*Citrus limon*	Portugal, Monchique	Twig dieback	*MF418408*	*MF418568*	*MF418328*	*MF418487*	*MF418242*
	CPC 28071	*Citrus limon*	Spain, Algemesi	Twig dieback	*MF418409*	*MF418569*	*MF418329*	*MF418488*	*MF418243*
	CPC 28072	*Citrus limon*	Spain, Algemesi	Twig dieback	*MF418410*	*MF418570*	*MF418330*	*MF418489*	*MF418244*
	CPC 28073	*Citrus reticulata*	Spain, Algemesi	Twig dieback	*MF418411*	*MF418571*	*MF418331*	*MF418490*	*MF418245*
	CPC 28074	*Citrus reticulata*	Spain, Algemesi	Twig dieback	*MF418412*	*MF418572*	*MF418332*	*MF418491*	*MF418246*
	CPC 28077	*Citrus limon*	Spain, Algemesi	Twig dieback	*MF418413*	*MF418573*	*MF418333*	*MF418492*	*MF418247*
	CPC 28079	*Citrus reticulata*	Spain, Algemesi	Twig dieback	*MF418414*	*MF418574*	*MF418334*	*MF418493*	*MF418248*
	CPC 28081 = CBS 142548	*Citrus reticulata*	Spain, Algemesi	Twig dieback	*MF418415*	*MF418575*	*MF418335*	*MF418494*	*MF418249*
	CPC 28163	*Microcitrus australasica*	Italy, Catania	Twig dieback	*MF418416*	*MF418576*	*MF418336*	*MF418495*	*MF418250*
	CPC 31135	*Citrus limon*	Malta, Gozo	Branch canker	*MF418417*	*MF418577*	*MF418337*	*MF418496*	*MF418251*
	CPC 31159	*Citrus sinensis*	Malta, Zurrieq	Branch canker	*MF418418*	*MF418578*	*MF418338*	*MF418497*	*MF418252*
D. helianthi	CBS 344.94	*Helianthus annuus*	-	-	KC343114	KC344082	KC343598	KC343840	KC343356
	CBS 592.81	*Helianthus annuus*	Serbia	-	KC343115	KC344083	KC343599	KC343841	JX197454
D. hongkongensis	**CBS 115448**	*Dichroa febrifuga*	China	-	KC343119	KC344087	KC343603	KC343845	KC343361
D. inconspicua	**CBS 133813**	*Maytenus ilicifolia*	Brazil	-	KC343123	KC344091	KC343607	KC343849	KC343365
D. infertilis	CBS 199.39	Unknown	Italy	-	KC343051	KC344019	KC343535	KC343777	KC343293
	CBS 230.52	*Citrus sinensis*	Suriname	-	KC343052	KC344020	KC343536	KC343778	KC343294

Table 1. (Continued).

Species	Culture no.[1]	Host	Locality	Associated symptoms	GenBank no.[2]				
					ITS	*tub2*	*his3*	*tef1*	*cal*
	CPC 20322	*Glycine max*	Brazil	-	KC343053	KC344021	KC343537	KC343779	KC343295
D. limonicola	CPC 27869	*Citrus limon*	Malta, Gozo	Trunk canker	*MF418419*	*MF418579*	*MF418339*	*MF418498*	*MF418253*
	CPC 27871	*Citrus limon*	Malta, Gozo	Trunk canker	*MF418420*	*MF418580*	*MF418340*	*MF418499*	*MF418254*
	CPC 27879	*Citrus limon*	Malta, Gozo	Branch canker	*MF418421*	*MF418581*	*MF418341*	*MF418500*	*MF418255*
	CPC 28200 = CBS 142549	*Citrus limon*	Malta, Gozo	Branch canker	*MF418422*	*MF418582*	*MF418342*	*MF418501*	*MF418256*
	CPC 31137 = CBS 142550	*Citrus limon*	Malta, Zurrieq	Branch canker	*MF418423*	*MF418583*	*MF418343*	*MF418502*	*MF418257*
D. melitensis	**CPC 27873 = CBS 142551**	*Citrus limon*	Malta, Gozo	Branch canker	*MF418424*	*MF418584*	*MF418344*	*MF418503*	*MF418258*
	CPC 27875 = CBS 142552	*Citrus limon*	Malta, Gozo	Branch canker	*MF418425*	*MF418585*	*MF418345*	*MF418504*	*MF418259*
D. multigutullata	**ICMP20656**	*Citrus grandis*	China	-	KJ490633	KJ490454	KJ490575	KJ490512	-
D. novem	CBS 127270	*Glycine max*	Croatia	-	KC343156	KC344124	KC343640	KC343882	KC343398
	CBS 127271	*Glycine max*	Croatia	-	KC343157	KC344125	KC343641	KC343883	KC343399
	CPC 26188 = CBS 142553	*Citrus japonica*	Italy, Messina	Twig dieback	*MF418426*	*MF418586*	*MF418346*	*MF418505*	*MF418260*
	CPC 28165 = CBS 142554	*Citrus aurantiifolia*	Italy, Catania	Twig dieback	*MF418427*	*MF418587*	*MF418347*	*MF418506*	*MF418261*
	CPC 28167	*Citrus aurantiifolia*	Italy, Catania	Twig dieback	*MF418428*	*MF418588*	*MF418348*	*MF418507*	*MF418262*
	CPC 28169	*Citrus aurantiifolia*	Italy, Catania	Twig dieback	*MF418429*	*MF418589*	*MF418349*	*MF418508*	*MF418263*
D. ovalispora	**ICMP20659**	*Citrus limon*	China	-	KJ490628	KJ490449	KJ490570	KJ490507	-
D. pseudomangiferae	**CBS 101339**	*Mangifera indica*	Dominican Republic	-	KC343181	KC344149	KC343665	KC343907	KC343423
D. pseudophoenicicola	**CBS 462.69**	*Phoenix dactylifera*	Spain	-	KC343184	KC344152	KC343668	KC343910	KC343426
D. rudis	CBS 113201	*Vitis vinifera*	Portugal	-	KC343234	KC344202	KC343718	KC343960	KC343476
D. saccarata	**CBS 116311**	*Protea repens*	South Africa	-	KC343190	KC344158	KC343674	KC343916	KC343432
D. sojae	FAU 635	*Glycine max*	USA	-	KJ590719	KJ610875	KJ659208	KJ590762	-
D. sojae	ZJUD68	*Citrus unshiu*	China	-	KJ490603	KJ490424	KJ490545	KJ490482	-
D. sterilis	**CBS 136969**	*Vaccinium corymbosum*	Italy	-	KJ160579	KJ160528	*MF418350*	KJ160611	KJ160548
D. subclavata	**ICMP20663**	*Citrus unshiu*	China	-	KJ490630	KJ490451	KJ490572	KJ490509	-
D. unshiuensis	**CGMCC3.17569**	*Citrus unshiu*	China	-	KJ490587	KJ490408	KJ490529	KJ490466	-
Diaporthella corylina	**CBS 121124**	*Corylus* sp.	China	-	KC343004	KC343972	KC343488	KC343730	KC343246

[1] CPC: Culture collection of P.W. Crous, housed at Westerdijk Fungal Biodiversity Institute; CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CGMCC: China, General Microbiological Culture Collection, Beijing, China; FAU: Isolates in culture collection of Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, MD, USA; ICMP: International Collection of Microorganisms from Plants, Landcare Research, Auckland, New Zealand; ZJUD, *Diaporthe* strains in Zhejiang University, China. Ex-type and ex-epitype cultures are indicated in **bold**.

[2] ITS: internal transcribed spacers 1 and 2 together with 5.8S nrDNA; *tub2*: partial beta-tubulin gene; *his3*: histone3; *tef1*: partial translation elongation factor 1-α gene; *cal*: partial calmodulin gene. Sequences generated in this study indicated in *italics*.


CBS 121124 Corylus sp. China
CBS 113201 Vitis vinifera Portugal
CBS 136969 Vaccinium corymbosum Italy
ICMP20657 Citrus limon China
CBS 134240 Citrus unshiu China
ICMP20662 Citrus unshiu China
CBS 117499 Aspalathus linearis South Africa
CBS 111592 Heracleum sphondylium Austria
CBS 127271 Glycine max Croatia
CPC 26188 Fortunella margarita Italy
CPC 28169 Citrus aurantiifolia Italy
CBS 127270 Glycine max Croatia
CPC 28167 Citrus aurantiifolia Italy
CPC 28165 Citrus aurantiifolia Italy
CBS 344.94 Heliantus annuus -
CBS 592.81 Heliantus annuus Serbia
CGMCC3.17569 Citrus unshiu China
CPC 20322 Glycine max Brazil
CBS 230.52 Citrus sinensis Suriname
CBS 199.39 Italy
ICMP20659 Citrus limon China
ZJUD73 Citrus unshiu China
FAU635 Glycine max USA
ZJUD68 Citrus unshiu China
CBS 134237 Citrus reticulata China
CBS 135422 Citrus sp. USA
CBS 134239 Citrus sinensis USA
ICMP20663 Citrus unshiu China
CBS 439.82 Cotoneaster sp. Scotland
CBS 134242 Citrus sp. China
ICMP20656 Citrus grandis China
ICMP20654 Citrus grandis China
CBS 115448 Dichroa febrifuga China
CBS 161.64 Areca catechu India
CBS 535.75 Citrus sp. Suriname
CBS 462.69 Phoenix dactylifera Spain
CBS 114979 Arenga engleri Honk Kong
CBS 101339 Mangifera indica Dominican Republic
CPC 27875 Citrus limon Malta
CPC 27873 Citrus limon Malta
CPC 27869 Citrus limon Malta
CPC 27871 Citrus limon Malta
CPC 27879 Citrus limon Malta
CPC 28200 Citrus limon Malta
CPC 31137 Citrus limon Malta
CBS 116311 Protea repens South Africa
CBS 133813 Maytenus ilicifolia Brazil
CBS 137020 Citrus limon Spain
CPC 26927 Citrus maxima Greece
CPC 31159 Citrus sinensis Malta
CPC 27949 Citrus sinensis Portugal
CPC 28043 Citrus limon Portugal
CPC 28041 Citrus limon Portugal
CPC 28047 Citrus limon Portugal
CPC 28033 Citrus sinensis Portugal
CPC 28045 Citrus limon Portugal
CPC 28073 Citrus reticulata Spain
CPC 28074 Citrus reticulata Spain
CPC 26913 Citrus limon Greece
CPC 26923 Citrus maxima Greece
CPC 27027 Citrus limon Italy
CPC 27167 Citrus paradisi Italy
CPC 27895 Fortunella margarita Malta
CPC 31135 Citrus limon Malta
CBS 135430 Citrus limon USA
CPC 27877 Citrus limon Malta
CPC 27896 Fortunella margarita Malta
CPC 28035 Citrus paradisi Portugal
CPC 28081 Citrus reticulata Spain
CPC 26873 Citrus reticulata Greece
CPC 26971 Citrus mitis Italy
CPC 28039 Citrus limon Portugal
CPC 28163 Microcitrus australasica Italy
CBS 111553 Foeniculum vulgare Spain
CBS 111554 Foeniculum vulgare Spain
CBS 123208 Foeniculum vulgare Portugal
CBS 123209 Foeniculum vulgare Portugal
CPC 26885 Citrus bergamia Greece
CPC 27959 Citrus sinensis Portugal
CPC 28079 Citrus reticulata Spain
CPC 27037 Citrus paradisi Italy
CPC 27859 Citrus paradisi Malta
CPC 26953 Citrus bergamia Greece
CPC 27897 Fortunella Margarita Malta
CPC 27041 Citrus sinensis Italy
CPC 27901 Citrus limon Malta
CPC 28071 Citrus limon Spain
CPC 26439 Citrus reticulata Italy
CPC 26863 Citrus maxima Greece
CPC 27756 Citrus limon Italy
CPC 26184 Citrus maxima Italy
CPC 26194 Citrus sinensis Italy
CPC 26365 Citrus limon Italy
CPC 26441 Citrus reticulata Italy
CPC 26461 Citrus reticulata Italy
CPC 27832 Citrus sinensis Italy
CPC 27833 Citrus sinensis Italy
CBS 187.27 Camellia sinensis Italy
CPC 26883 Citrus maxima Greece
CPC 26967 Citrus mitis Italy
CPC 27033 Citrus mitis Italy
CPC 27898 Fortunella margarita Malta
CPC 27903 Citrus limon Malta
CPC 28077 Citrus limon Spain
CPC 27945 Citrus paradisi Portugal
CPC 27947 Citrus sinensis Portugal
CPC 27950 Citrus sinensis Portugal
CPC 28072 Citrus limon Spain
CPC 26170 Citrus sinensis Italy
CPC 26465 Citrus limon Italy
CPC 26963 Citrus paradisi Italy
CPC 27079 Citrus limon Italy
CPC 27831 Citrus sinensis Italy
CPC 27834 Citrus sinensis Italy
CPC 27835 Citrus sinensis Italy
CPC 27836 Citrus sinensis Italy
CPC 27837 Citrus sinensis Italy
CPC 27029 Citrus sinensis Italy
CPC 27821 Citrus reticulata Italy
CPC 27075 Citrus limon Italy
CPC 27850 Citrus sinensis Italy
CPC 27852 Citrus sinensis Italy
CBS 136972 Vaccinium corymbosum Italy
Diaporthella corylina
Diaporthe rudis
D. sterilis
D. biguttulata
D. citriasiana
D. discoidispora
D. cuppatea
D. angelicae
D. novem
D. helianti
D. unshiuensis
D. infertilis
D. ovalispora
D. endophytica
D. sojae
D. citri
D. subclavata
D. eres
D. citrichinensis
D. multiguttulata
D. biconispora
D. hongkongensis
D. arecae
D. pseudophoenicicola
D. arengae
D. pseudomangiferae
D. melitensis
D. limonicola
D. saccarata
D. incospicua
D. cytosporella
D. foeniculina
D. baccae
0.92/100
2X
1/99
1/72
1/99
0.98/88
0.99/92
1/97
1/91
1/97
1/89
1/60
1/99
1/64
0.58/-
1/54
1/-
0.99/-
0.8/-
0.9/56
1/-
0.83/-
1/99
1/85
0.65/-
0.88/-
0.96/50
0.83/-
0.76/87
0.75/57
0.57/-
0.89/-
0.96/97
0.05

Table 2. *Diaporthe* species associated with citrus and their morphological characteristics.

Species	Conidiomata (µm)	Conidiophores (µm)	Alpha conidia (µm)	Beta conidia (µm)	References
D. arecae	up to 400	15–40 × 1.5–3	6–10 × 2–3	-	Gomes *et al.* (2013)
D. baccae	up to 650	20–57 × 2–3	7–9 × 2–3	20–24 × 1–2	Lombard *et al.* (2014)
D. biconispora	145–185	12–35.5 × 1.6–2.6	6–10.5 × 2–3.5	-	Huang *et al.* (2015)
D. biguttulata	up to 300	5.8–16.9 × 1.3–2.3	5.7–7.8 × 2.5–2.9	23.7–31.6 × 0.9–1.6	Huang *et al.* (2015)
D. citri	200–250	10–15 × 1–2	7.6–10.2 × 3–4.2	-	Udayanga *et al.* (2014b)
D. citriasiana	up to 627	3.5–10.5 × 1–2	10.5–15 × 4–6.5	24–42 × 1–2	Huang *et al.* (2013)
D. citrichinensis	up to 435	9–19.5 × 1.5–3	5.5–9 × 1.5–2.5	27.5–40 × 1–1.5	Huang *et al.* (2013)
D. cytosporella	150–200	7–18 × 1–2	8–9 × 2.6–3.2	-	Udayanga *et al.* (2014b)
D. discoidispora	200–118	8.9–23.4 × 1.3–2.7	5.6–8 × 2.1–3.2	21.2–38.7 × 0.9–1.6	Huang *et al.* (2015)
D. endophytica (sterile)	-	-	-	-	Gomes *et al.* (2013)
D. eres	200–250	10–15 × 2–3	6.5–8.5 × 3–4	22–28 × 1–1.5	Udayanga *et al.* (2014a)
D. foeniculina	400–700	9–15(–18) × 1–2	8.5–9 × 2.3–2.5	22–28 × 1.4–1.6	Udayanga *et al.* (2014b)
D. hongkongensis	up to 200	5–12 × 2–4	6–7 × 2.5	18–22 × 1.5–2	Gomes *et al.* (2013)
D. infertilis (sterile)	-	-	-	-	This study
D. limonicola	up to 670	5–20 × 1.5–4	5.5–8.5 × 1.5–2.5	15–26.5 × 1–2	This study
D. melitensis	up to 650	5–15 × 1.5–5.5	4.5–7 × 1.5–3	-	This study
D. multigutullata	up to 358	9.8–14.8 × 1.3–3.6	8–12.6 × 4.2–6	-	Huang *et al.* (2015)
D. novem	up to 580	5.3–10.4 × 1.9–3.2	6.3–8.9 × 1.9–2.5	26.4–37.7 × 1–1.3	Santos *et al.* (2011)
D. ovalispora	up to 242	9.5–21.6 × 1.6–3.6	6.1–7.9 × 2.7–3.8	-	Huang *et al.* (2015)
D. sojae	200–250	12–16 × 2–4	5.3–7.3 × 2–3	-	Udayanga *et al.* (2015)
D. subclavata	-	14.2–27.3 × 1.6–2.6	5.5–7.2 × 2.2–2.9	-	Huang *et al.* (2015)
D. unshiuensis	up to 152	14.3–24.2 × 1.4–2.6	5.2–7.5 × 2–3.9	-	Huang *et al.* (2015)

TAXONOMY

Morphological observations, supported by phylogenetic inference, were used to identify three known species (*D. baccae*, *D. foeniculina,* and *D. novem*), and to recognize three new species described here (Table 2). One species (represented by three isolates) was sterile in culture, and is therefore characterized by DNA sequence data (Gomes *et al*. 2013).

Diaporthe infertilis Guarnaccia & Crous, **sp. nov.**

MycoBank MB821727
(Fig. 3)

Etymology: Named after its sterile growth in culture.

Diagnosis: *Diaporthe infertilis* differs from its closest phylogenetic neighbour, *D. ovalispora*, in 26 unique fixed alleles in ITS locus, 68 in *tef1*, 30 in *tub2* and 48 in *his3* based on the alignments deposited in TreeBASE.

Type: **Suriname**: Paramaribo, from decaying fruit of *Citrus sinensis*, Apr. 1932, *N.J. van Suchtelen* (CBS H-23179 – holotype; CBS 230.52 – culture ex-type).

Description: *Culture characteristics*: Colony on MEA covering the entire plate after 10 d, pale luteous with abundant white compact aerial mycelium in fluctuating rings. On OA and PDA at first white, becoming cream to yellowish, flat, with dense and felted mycelium, reverse pale brown with brownish dots with age. Cultures sterile.

Notes: Three isolates clustered in a clade distinct from species of *Diaporthe* known from DNA sequence data. One strain (CPC 20322) was differentiated from the other two (CBS 199.39, CBS 230.52) by unique fixed alleles in four loci based on alignments of the separate loci deposited in TreeBASE: *tef1* positions 115 (C), 261 (indel), 314 (G), 395 (C); *tub2* positions 123 (C), 631 (G); *cal* positions 132 (T), 207 (A), 210 (T), 256 (T), 259 (T), 262 (A), 364 (G), 366 (A), 438 (G), 439 (G), 448 (C); *his3* positions 201 (A), 438 (A), 448 (T), 450 (A). Gomes *et al.* (2013) tentatively referred to this clade as *D. citri*. However, after a molecular re-assessment of many *Diaporthe* species, *D. citri* is restricted to a different clade of citrus isolates (Udayanga *et al*. 2014b). We therefore describe *D. infertilis* as a new species for this clade.

Fig. 2. Consensus phylogram of 3 762 trees resulting from a Bayesian analysis of the combined ITS, *tub2*, *his3*, *tef1* and *cal* sequence. Bootstrap support values and Bayesian posterior probability values are indicated at the nodes. The asterisk symbol (*) represents full support (1/100). Substrate and country of origin are listed next to the strain numbers. The newly recognized species are in red. The tree was rooted to *Diaporthella corylina* (CBS 121124).

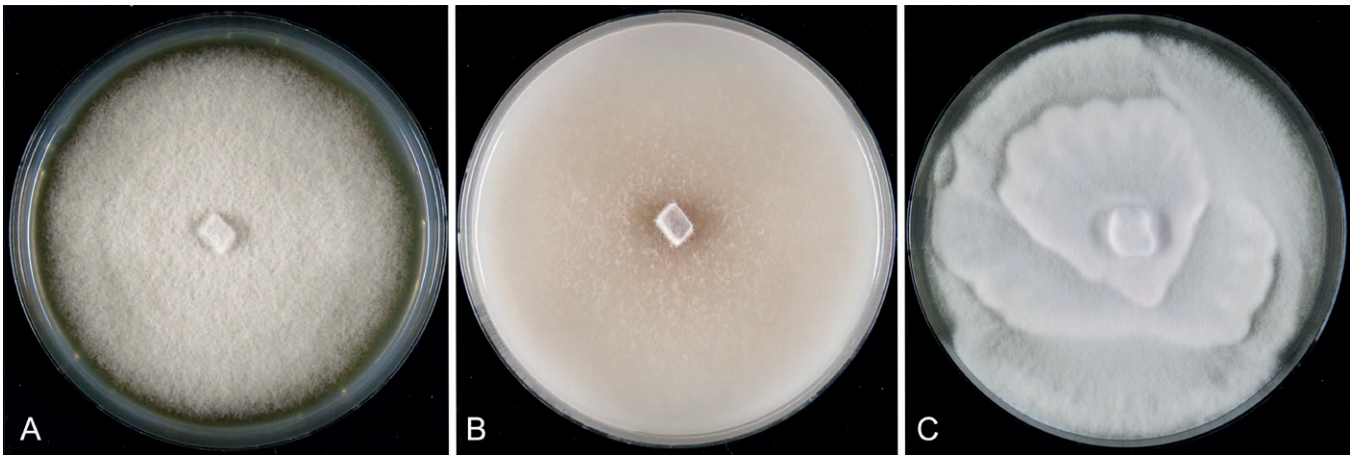

Fig. 3. *Diaporthe infertilis* (CBS 230.52). **A–C.** Colonies after 7 d at 21 °C on MEA, OA and PDA, respectively.

Additional material examined: **Brazil**: from seeds of *Glycine max*, *A. Almeida* (culture LGMF946 = CPC 20322). – **Italy**: from unknown host, *G. Goidanich* (CBS 199.39).

Diaporthe limonicola Guarnaccia & Crous, **sp. nov.**
MycoBank MB821731
(Fig. 4)

Etymology: In reference to the occurrence on *Citrus limon*.
Diagnosis: *Diaporthe limonicola* can be distinguished from the closely related *D. pseudomangiferae* based on *tef1*, *tub2*, *his3* and *cal* loci (96 % in *tef1*, 96 % in *tub2*, 97 % in *his3*, and 96 % in *cal*). *Diaporthe limonicola* differs from *D. pseudomangiferae* in the shorter alpha conidia and in producing beta and gamma conidia.

Type: **Malta**: Gozo, from branch canker of *Citrus limon*, 11 Jul. 2016, *V. Guarnaccia* (CBS H-23126 – holotype; CBS 142549 = CPC 28200 – culture ex-type).

Description: *Conidiomata* pycnidial in culture on PNA, PDA, OA and MEA, solitary or aggregated, deeply embedded in

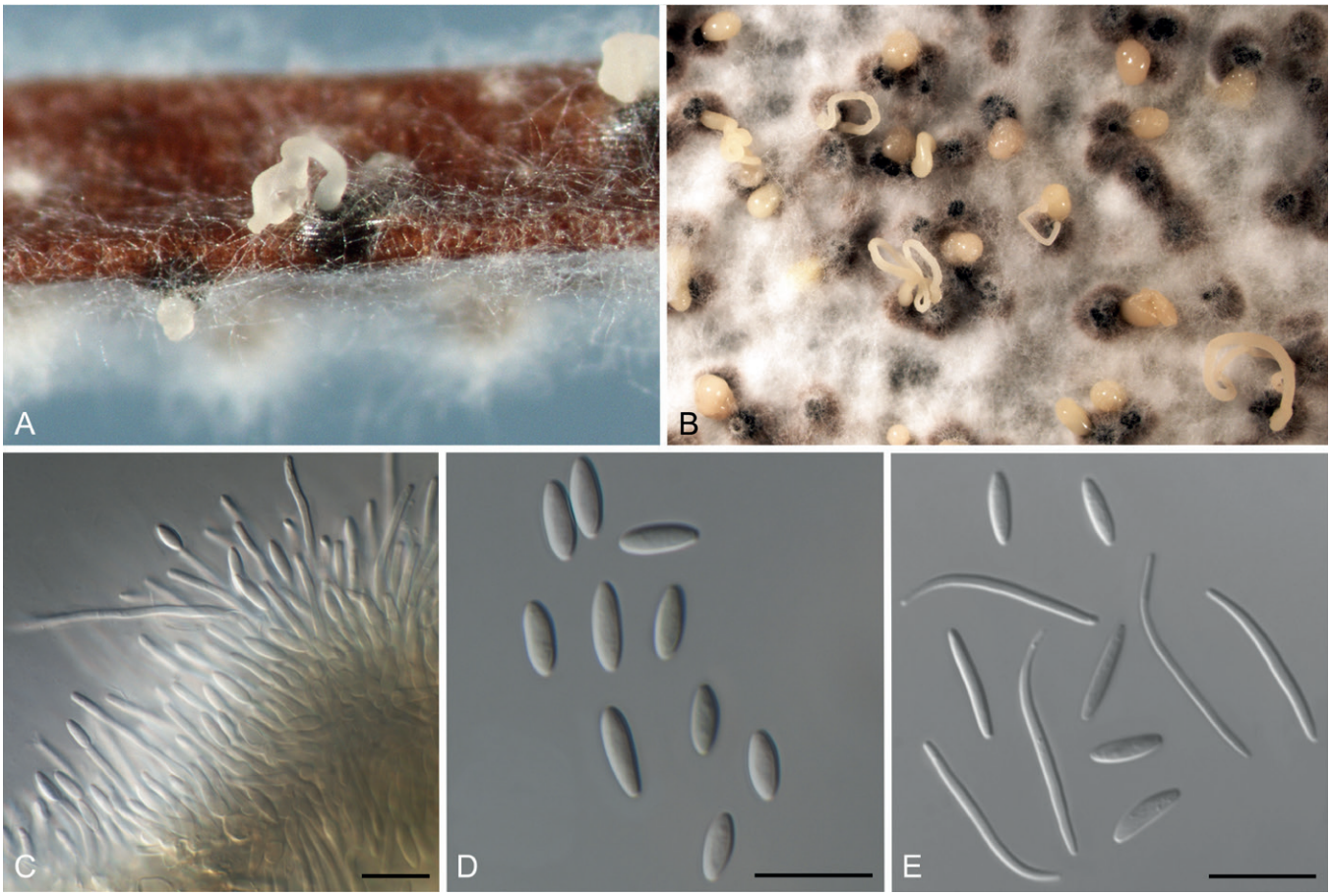

Fig. 4. *Diaporthe limonicola* (CBS 142549). **A.** Conidiomata sporulating on PNA. **B.** Conidiomata sporulating on OA. **C.** Conidiogenous cells. **D.** Alpha conidia. **E.** Alpha, beta and gamma conidia. Bars = 10 µm.

PDA, erumpent, dark brown to black, 250–670 µm diam, whitish translucent to cream conidial drops exuded from the ostioles. *Conidiophores* hyaline, smooth, 1-septate, densely aggregated, cylindrical, straight, 5–20 × 1.5–4 µm. *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 5–12 × 1–2 µm, tapered towards the apex. *Paraphyses* intermingled among conidiophores, hyaline, smooth, 1–3-septate, to 90 µm long, apex 1–2 µm diam. *Alpha conidia* unicellular, aseptate, fusiform, hyaline, mono- to biguttulate and acute at both ends, 5.5–8.5 × 1.5–2.5 µm, mean ± SD = 6.8 ± 0.6 × 2.1 ± 0.3 µm, L/B ratio = 2.8. *Beta conidia* hyaline, aseptate, eguttulate, filiform, curved, tapering towards both ends, 15–26.5 × 1–2 µm, mean ± SD = 22.7 ± 2.6 × 1.4 ± 0.3 µm, L/B ratio = 16.2. *Gamma conidia* hyaline, multiguttulate, fusiform to subcylindrical with an acute or rounded apex, 9–15.5 × 1–2 µm, mean ± SD = 10.7 ± 1.6 ×1.4 ± 0.2 µm, L/B ratio 7.6.

Culture characteristics: Colonies covering the medium within 1 wk at 21 °C, surface mycelium flattened, dense and felt-like. Colony on MEA and OA at first white, becoming cream to yellowish, flat, with dense and felted mycelium, reverse pale brown with brownish dots with age, with visible solitary or aggregated conidiomata at maturity. On PDA cream to smoke-grey, reverse pale brown.

Notes: *Diaporthe limonicola* was isolated from *Citrus limon* trunk cankers in two different islands of the Malta archipelago, where all the plants were affected. Five strains representing *D. limonicola* cluster in a well-supported clade, and appear most closely related to *D. pseudomangiferae* and *D. arengae*. *Diaporthe limonicola* can be distinguished based on *tef1*, *tub2*, *his3* and *cal* loci from *D. pseudomangiferae* (96 % in *tef1*, 96 % in *tub2*, 97 % in *his3*, and 96 % in *cal*), and from *D. arengae* (97 % in *tef1*, 98 % in *tub2*, 98 % in *his3*, and 96 % in *cal*). This species is phylogenetically close to but clearly differentiated from *D. melitensis* (described below) by 22 unique fixed alleles in ITS locus, 2 in *tef1* and 47 in *tub2*.

Morphologically, *D. limonicola* differs from *D. pseudomangiferae* in the shorter alpha conidia (5.5–8.5 *vs*. 7–9 µm) (Gomes *et al*. 2013) and the production of beta and gamma conidia, which are not known in *D. pseudomangiferae* (Gomes *et al*. 2013).

Additional material examined: **Malta**: Zurrieq, from branch canker of *Citrus limon*, 11 Jul. 2016, *V. Guarnaccia* (culture CBS 142550 = CPC 31137).

Diaporthe melitensis Guarnaccia & Crous, **sp. nov.**
MycoBank MB821732
(Fig. 5)

Etymology: Named after the country where it was collected, Malta (ancient Latin name, *Melita*).

Diagnosis: *Diaporthe melitensis* can be distinguished from the closely related *D. pseudomangiferae* by the ITS, *tef1*, *tub2*, *his3* and *cal* loci (98 % in ITS, 96 % in *tef1*, 97 % in *tub2*, 97 % in *his3*, and 96 % in *cal*). *Diaporthe melitensis* also differs from *D. pseudomangiferae* in the shorter alpha conidia.

Type: **Malta**: Gozo, from branch canker of *Citrus limon*, 22 Sep. 2015, *V. Guarnaccia* (CBS H-23127 – holotype; CBS 142551 = CPC 27873 – culture ex-type).

Description: *Conidiomata* pycnidial in culture on PNA, PDA, OA and MEA, solitary or aggregated, deeply embedded in the PDA, erumpent, dark brown to black, 250–650 µm diam, whitish translucent to yellowish conidial drops exuded from the ostioles. *Conidiophores* hyaline, smooth, 1-septate, densely aggregated, cylindrical, straight, 5–15 × 1.5–5.5 µm. *Conidiogenous cells* phialidic, hyaline, terminal, cylindrical, 6–12 × 1–3 µm, tapered towards the apex. *Paraphyses* not observed. *Alpha conidia* unicellular, aseptate, fusiform, hyaline, 1–4-guttulate with acute ends, 4.5–7 × 1.5–3 µm, mean ± SD = 5.9 ± 0.6 × 2.2 ± 0.4 µm, L/B ratio = 2.7. *Beta conidia* and *Gamma conidia* not observed.

Culture characteristics: Colonies covering the dish within 1 wk at 21 °C, surface mycelium flattened, dense and felt-like. Colony on MEA and OA at first white, becoming yellowish, flat, with dense and felted mycelium, reverse pale sepia with brownish dots with age, with visible solitary or aggregated conidiomata at maturity. On PDA cream to smoke-grey, reverse pale brown.

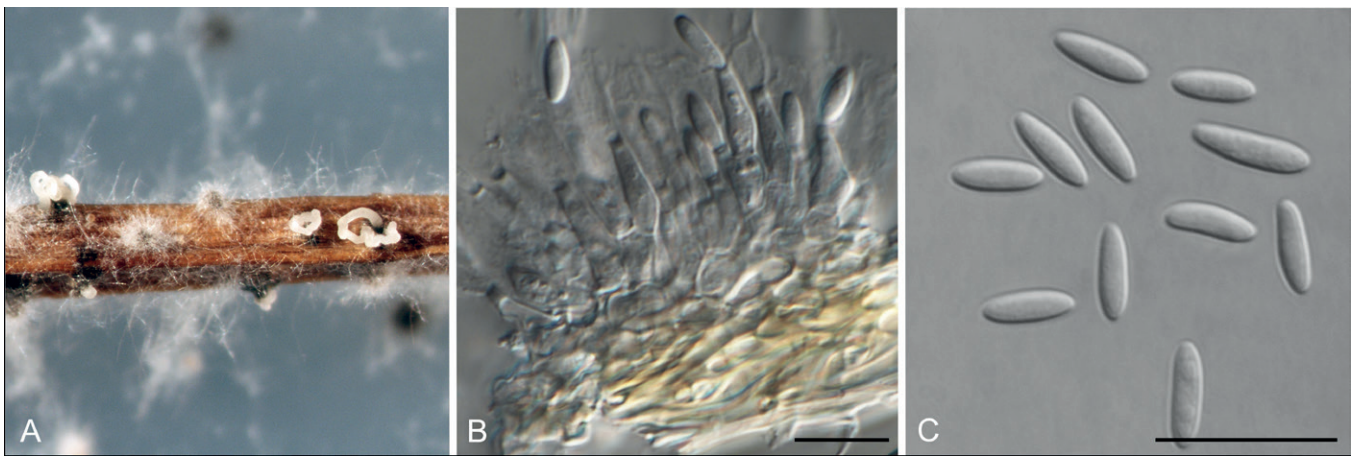

Fig. 5. *Diaporthe melitensis* (CBS 142551). **A.** Conidiomata sporulating on PNA. **B.** Conidiogenous cells. **C.** Alpha conidia. Bars = 10 µm.

Notes: *Diaporthe melitensis* was isolated from trunk samples of *Citrus limon* showing serious cankers in Gozo (Malta). The two strains representing *D. melitensis* cluster in a well-supported clade, and appear closely related to *D. pseudomangiferae* and *D. arengae*. This species is phylogenetically closely related to, but clearly differentiated from, *D. limonicola* (described above) by 22 different unique fixed alleles in ITS, *tef1* and *tub2* loci (22, 2, and 47 respectively) based on the alignments deposited in TreeBASE.

Morphologically *D. melitensis* differs from *D. pseudomangiferae* in the shorter alpha conidia (4.5–7 *vs*. 7–9 µm) (Gomes *et al*. 2013).

Additional material examined: **Malta**: Gozo, from branch canker of *Citrus limon*, 22 Sep. 2015, *V. Guarnaccia* (culture CBS 142552 = CPC 27875).

PATHOGENICITY

After 30 d all the isolates of the inoculated species induced lesions on most of the *Citrus* species tested. The inoculated twigs developed cankers similar to those detected in the field, and the fungi were successfully re-isolated, fulfilling Koch's postulates (Fig. 6). Cankers and internal discolouration were observed in correspondence to inoculation points. On the contrary, no symptoms were observed on the control plants. Clear differences in aggressiveness among the isolates and susceptibility of the *Citrus* species were observed: *D. limonicola* and *D. melitensis* caused the most serious symptoms with no difference among the hosts. *Diaporthe foeniculina* was weakly aggressive to each *Citrus* species. Similarly, *D. novem* was weakly aggressive on all the hosts except the orange clones, whilst *D. baccae* caused disease symptoms only on mandarin.

DISCUSSION

After a major screening of fungal diseases of citrus in Europe (Guarnaccia *et al*. 2017a, b, Sandoval-Denis *et al*. 2018), molecular phylogenetic and morphological analyses were used to evaluate the diversity of *Diaporthe* species in the Mediterranean basin, focusing on symptomatic plants.

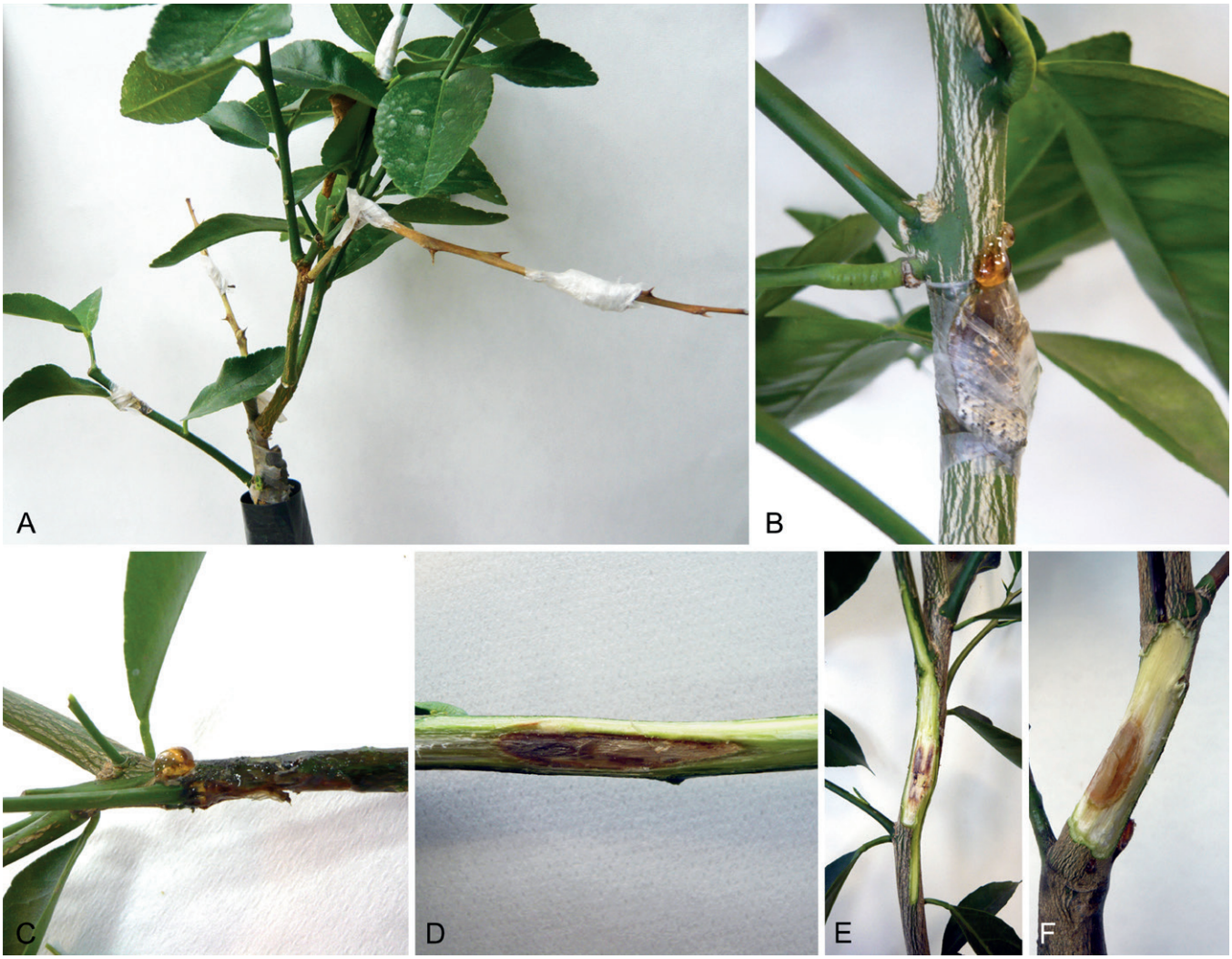

Fig. 6. Pathogenicity test of selected *Diaporthe* isolates on citrus plants after 30 d. **A.** Shoot blight of lime plants inoculated with *D. novem* (CPC 26188). **B**–**C.** Cankers with gummosis of lemon plants caused by *D. limonicola* and *D. melitensis* (CPC 28200, CPC 27873). **D**–**E.** Internal discoloration of mandarin twigs inoculated respectively with *D. melitensis* and *D. baccae* (CPC 27873, CPC 26170). **F.** Internal lesion of orange branch caused by *D. foeniculina* (CPC 28081).

Several *Diaporthe* species are well established in Europe (Thomidis & Michailides 2009, Santos *et al*. 2011, Lombard *et al.* 2014, Guarnaccia *et al.* 2016). *Diaporthe* species are also frequently associated with citrus diseases worldwide (Timmer *et al*. 2000, Huang *et al*. 2013), such as melanose and stem-end rot. Since the late 18th century these diseases have affected different citrus organs and also cause a sort of wood gummosis (Fawcett 1936, Timmer *et al*. 2000, Mondal *et al*. 2007). *Diaporthe citri* is considered a key pathogen of *Citrus* species and has been confirmed from Brazil, China, Korea, and New Zealand, and is also reported as widely spread throughout Asia, Australasia, and South America (Timmer *et al.* 2000, Mondal *et al*. 2007, Udayanga *et al*. 2014b). However, *D. citri* has never been reported from Europe, whilst *D. cytosporella* and *D. foeniculina* have been recently isolated from citrus in Spain (Udayanga *et al.* 2014b).

DNA sequence data are essential in resolving taxonomic questions, redefining species boundaries, and the accurate naming of species required for effective communication about plant pathogens. Thus, during the past decade, a polyphasic approach was used in several *Diaporthe* studies, revealing new species involved with citrus diseases and as endophytes and plant pathogens (Huang *et al*. 2013, 2015). Santos *et al.* (2017a) showed that species separation is better when five loci (ITS, *tef1*, *tub2*, *his3*, and *cal*) are simultaneously used to build the phylogeny of *Diaporthe* isolates.

Citrus crops are already compromised by a range of fungal pathogens other than *Diaporthe* (Vicent *et al.* 2007, Aiello *et al.* 2015, Guarnaccia *et al*. 2017a, Sandoval-Denis *et al.* 2018). Considering that no surveys for citrus diseases caused by *Diaporthe* had been performed in Europe, a large-scale investigation of *Diaporthe* species associated with citrus infections in Europe was needed. This study provides the first molecular characterization of *Diaporthe* diversity related to citrus production in Europe, combined with morphological characterisation.

Several citrus orchards, plant nurseries, private gardens and collections in five Mediterranean European countries were investigated. We further investigated different host plants in *Citrus*-allied genera such as *Microcitrus*, which is also economically important for fruit production.

Canker symptoms were frequently observed on several *Citrus* species in all countries investigated. Twigs showed wilting, dieback, wither-tip, and gummosis. Some orchards presented branch blight and trunk cankers associated with abundant gummosis. The most critical situation seen was in different lemon orchards in Malta, where the infections led to tree death. Melanose and stem-end rot were never observed.

We collected 79 *Diaporthe* strains. Phylogenetic analyses based on single and the combined five loci (ITS, *tef1*, *tub2*, *his3*, and *cal*), as well as morphological characters, revealed five *Diaporthe* species associated with infections on several *Citrus* species in Europe. We included in the analysis the closest taxa to the five *Diaporthe* species recovered in this study, based on BLAST searches of NCBI's GenBank nucleotide database. The final phylogenetic tree distinguished two newly described species (*D. limonicola* and *D. melitensis*) and three known species (*D. baccae*, *D. foeniculina*, and *D. novem)*. Moreover, a known clade represented by three strains (CBS 199.39, CBS 230.52, CPC 20322), previously named *D. citri*, appeared in our final tree. However, this clade also required a separate name as *D. citri s. str.* is restricted to the pathogen causing melanose and stem-end rot of citrus fruit (Udayanga *et al.* 2014b). Thus, in this study we have described these three isolates as *D. infertilis*. Based on sampling in this study, *D. citri* appears to be absent in Europe as previously reported by Udayanga *et al.* (2014b).

Huang *et al.* (2015) obtained two separate groups of citrus isolates within the *D. arecae* complex, which were either not well supported or non-monophyletic based on a four-locus phylogenetic analysis. However, our analysis based on five loci, combined with morphological observations, clearly separated both *D. limonicola* and *D. melitensis* from *D. pseudomangiferae* and *D. areangae*, the most closely related species, and from other species in the *D. arecae* complex such as *D. podocarpi-macrophylli* and *D. xishuangbanica* (Gao *et al*. 2017). Morphologically, *D. limonicola* and *D. melitensis* differ from *D. pseudomangiferae* in the shorter alpha conidia. Moreover, *D. limonicola* is the only taxon among these species to produces beta and gamma conidia.

Diaporthe foeniculina was the predominant species found in all the Mediterranean countries sampled, but its pathogenicity on *Citrus* was unknown (Udayanga *et al.* 2014b). Recently, Lombard *et al*. (2014) described *D. baccae* as a new species associated with *Vaccinium corymbosum* cankers in Italy. Similarly, we found this species associated with twig, branch and trunk cankers of citrus in Italy. *Diaporthe novem* was isolated for the first time from infected citrus plants in our study, where it was found associated with twig dieback of *C. japonica* (kumquat) and *C. aurantiifolia* (lime) in Italy. Moreover, the newly described species were isolated from devastated lemon plants in several orchards on Malta: *D. limonicola* was recovered from symptomatic trunks and branches, whilst *D. melitensis* was isolated only from branches. They were isolated separately and from the same affected sample. Colonization of the same host plant by diverse *Diaporthe* species appears to be frequent as previously reported (Crous & Groenewald 2005, Van Niekerk *et al*. 2005, Thompson *et al.* 2011).

Our results reveal a large diversity of *Diaporthe* species spanning several clades and species complexes, associated with citrus wood cankers in European countries. These include *D. baccae*, *D. infertilis*, *D. novem*, and the two newly described species. In total, 22 *Diaporthe* species are now confirmed as associated with citrus.

Pathogenicity of the species isolated from citrus samples collected in Europe was tested on healthy plants of lemon, lime, mandarin, and two clones of *Citrus sinensis* ('New Hall' and 'Tarocco Meli'). All of the *Diaporthe* species tested caused lesions to develop on twigs. Recently, *D. foeniculina* (syn. *D. neotheicola*) has been reported as causing disease in many other hosts: shoot blight of persimmon in Australia (Golzar *et al*. 2012), kiwi-fruit disease in Greece (Thomidis *et al*. 2013), and avocado branch cankers (Guarnaccia *et al*. 2016). This species evidently has the ability to infect a wide range of fruits and plant hosts as an opportunistic pathogen. *Diaporthe foeniculina* (as "*D. foeniculacea*" in Gomes *et al*. 2013) proves to be a pathogen with a broad host range amongst temperate woody plants and fruit trees. In our study, *D. foeniculina* was

isolated from symptomatic plants of eight *Citrus* species (*C. bergamia*, *C. japonica*, *C. limon*, *C. maxima*, *C. mitis*, *C. paradisi*, *C. reticulata,* and *C. sinensis*) and also *Microcitrus australasica*. In the pathogenicity tests, it was weakly aggressive, but produced lesions on each species tested.

These results demonstrate a cross-infection potential of multiple *Diaporthe* species on different *Citrus* species, as previously reported (Lombard *et al*. 2014, Guarnaccia *et al.* 2016). *Diaporthe limonicola* and *D. melitensis* caused prominent symptoms in all the citrus species inoculated, and because they were isolated from plants with severe disease symptoms, these species can be considered as potentially major new pathogens of *Citrus limon*. *Diaporthe baccae* caused symptoms only on mandarin, while *D. novem* infected lime, lemon, and mandarin plants. Both of these species seemed to be weakly aggressive, with different host susceptibility and known distribution. These fungi merit adding to the list of fungal taxa causing citrus cankers worldwide (Adesemoye *et al*. 2014, Mayorquin *et al*. 2016, Sandoval-Denis *et al.* 2018).

This study provides the first overview of *Diaporthe* diversity associated with cankers of citrus plants in Europe, and includes information on their pathogenicity. Two of the new species described were established as causal agents of a devastating disease of lemon plants, inducing branch and trunk cankers that lead to plant death. The present study also appears to represent the first reports of *D. baccae* and *D. novem* associated with citrus disease in Europe. Despite the worldwide distribution and economical importance of citrus, knowledge of the fungal species associated with *Citrus* species is still incomplete. Further studies are required in order to fully elucidate the host range, specificity, and global distribution of *Diaporthe* species, as well as other fungi causing cankers of citrus plants.

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